IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#19

Attorney Docket No.:	**SJ-0005**
Inventors:	**Danks et al.**
Serial No.:	**09/595,682**
Filing Date:	**June 16, 2000**
Examiner:	**Celine X. Qian**
Group Art Unit:	**1636**
Title:	**Compositions and Methods for Sensitizing and Inhibiting Growth of Human Tumor Cells**

DECLARATION

1. We Mary K. Danks, and Philip M. Potter are co-inventors of the above referenced U.S. Patent Application Serial No. 09/595,682 filed June 16, 2000. On the basis of our qualifications, we submit that we are experts in the fields pertaining to the subject matter claimed in the above-captioned application, including the field of gene therapy as it pertains to the use of a carboxylesterase to metabolize CPT-11, and we are qualified to speak of the skill and knowledge of those of ordinary skill in these arts.

2. We have read and carefully studied the Office Action dated July 16, 2002. We have further read and carefully studied the previous Office Action issued on January 29, 2002 to the extent necessary to fully understand the Examiner's enablement rejection.

3. Our invention referenced above, teaches a method for sensitizing tumor cells to a chemotherapeutic prodrug comprising transfecting selected tumor cells with a composition comprising an isolated polynucleotide encoding a carboxylesterase (CE). In accordance with our invention, if sufficient CE levels can be achieved in tumor cells, then regressions will occur following CPT-11 treatment *in vivo*.

4. The following studies demonstrate that expression of CE and CPT-11 activation can be achieved *in vivo* following administration of an adenovirus containing CE.

5. Human tumor xenografts were injected with replication-deficient adenovirus containing the rabbit liver CE cDNA under control of the RSV promoter in accordance with the methods outline in the specification on page 40 line 20 - page 42 line 12. Significantly increased levels of CE activity were detected in the tumors up to 11 days following viral administration.

6. As shown in the attached Figure 1, after intratumoral injection of Rh30 human rhabdomyosarcoma xenografts with 5 X 10^9 pfu of adenovirus, the levels of CE activity increase reaching a maximum of 81 μmoles/min/mg on day 4 after administration. This level is about six fold higher than that observed in xenografts injected with a control virus lacking the rabbit liver CE cDNA. Since we have demonstrated that the sensitivity of cells to CPT-11 is directly proportional to the levels of exogenously expressed CPT-11 activating CEs, these tumor cells would be sensitized to the drug.

7. We performed studies using replication-competent adenovirus containing rabbit liver CE (Onyx 713). Following intratumoral injection of mice bearing A549 human tumor xenografts with 1 x 10^9 pfu of Onyx 713, CE activity peaked on day 4, and was about six fold greater than that observed from tumors excised from mice injected with control virus lacking the CE cDNA (see Figure 2 attached).

8. The intratumoral levels of SN-38 were approximately two fold greater in mice injected with virus containing rCE as shown in Figure 3 (attached). This finding was consistent with the enhanced activation of CPT-11 *in vivo*. Since CPT-11 has a remarkably steep dose-response curve, a two fold increase in SN-38 concentration is sufficient to make a difference between no response and a complete cure in mice bearing human tumor xenografts.

9. Survival assays using human tumors grown in nude mice verified that a six fold increase in levels of CE activity in xenografts was sufficient to induce an antitumor effect. As shown in the attached Figure 4, mice bearing C33-A human tumor xenografts injected with Onyx 713 virus intravenously in combination with CPT-11 demonstrate a 40% long term survival compared to no survivors for mice injected with CPT-11 alone.

10. In another experiment, mice containing human U373MG tumors derived from a glioblastoma cell line, were injected with 5 X 10^9 pfu of either AdCMVrCE or AdRSVrCE. These vectors both contain

the rabbit CE coding sequence under control of either the CMV or RSV promoter, respectively. Tumors were harvested at the indicated time points, and the levels of CE activity and CPT-11 conversion were determined from sonicates of whole xenografts. As shown in Figure 5, the levels of CE activity in tumors injected with AdCMVrCE were dramatically increased, approximately 70 fold greater than control tumors at 2 days.

11. Figure 6 shows that CPT-11 activation by these xenografts was also significantly greater than controls. Additionally, levels of CE expression were maintained for as long as 10 days. Whilst this is clearly not an optimized system, it is apparent that levels of CE activity sufficient to induce tumor responses in combination with CPT-11 can be achieved following direct intratumoral injection of adenovirus.

12. We are familiar with the results of Clinical trials involving gene therapy protocols in cancer patients as listed in Table 1. Over 100 patients have been enrolled in studies using virus directed enzyme prodrug therapy (VDEPT) approaches with plasmid, adenoviral or retroviral delivery of prodrug-activating enzymes. The studies listed in Table 1 included both replication-deficient and competent adenovirus, indicating that transient gene expression afforded by either vector is suitable for this procedure. Measurable antitumoral responses were documented in these studies. Even though these studies were published after our priority date, no new developments subsequent to that date were relied upon in these clinical trial studies.

13. Our studies outlined above, especially the results achieved in the experiment outlined in paragraphs 10 and 11, show that adenovirus is a suitable vector for delivery of CE *in vivo*. Furthermore, we demonstrate that intratumoral and intravenous administration of recombinant adenovirus are suitable delivery methods. Since we believe that short term, high level expression of CE is optimal for VDEPT with CPT-11, adenovirus provides the most suitable delivery vehicle to achieve this level of *in vivo* gene expression. It is our opinion that a person of ordinary skill in the art would not be required to perform undue research or experimentation to successfully apply the teachings found in our present application in an in vivo gene therapy setting.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Mary K. Danks

Mary K. Danks

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Table 1. Reported results for clinical trials using VDEPT protocols

Enzyme	Delivery	Drug	Disease	No. of patients	Reported data/comment	ref
Cytosine deaminase	Plasmid	5-fluorocytosine	Breast cancer	12	Targeted expression was observed in 90% of cells	(1)
HSVtk	Adenovirus	Ganciclovir	Mesothelioma	21	HSV expression was detected in 11 patients	(2)
HSVtk	Adenovirus	Ganciclovir	Prostate cancer	18	3 patients showed 50% reduction in serum PSA levels	(3)
HSVtk	Adenovirus	Acyclovir	Ovarian cancer	10	30% increase in overall survival	(4)
HSVtk	Retrovirus	Ganciclovir	Glioblastoma	48	Survival rate of 27% at 12 months	(5, 6)

In addition, there are currently numerous VDEPT clinical protocols open (as indicated by the Clinical Trials in Human Gene Transfer database) for a variety of cancers including bladder and colon, in addition to those listed above.

References

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Figure legends

Figure 1. CE activity in Rh30 xenografts following injection with either vector control adenovirus (AdVC), or adenovirus containing the rabbit liver CE cDNA (AdRSVrCE). 5 x 10^9 pfu of virus was administered intratumorally, and CE activity was determined at the times indicated from xenografts removed from the mice. Enzyme activity was determined using o-nitrophenyl acetate as a substrate.

Figure 2. CE activity in A549 xenografts following injection of replication competent adenovirus. Onyx O15 is a control virus and Onyx 713 contains the rabbit liver CE cDNA. 1 x 10^9 pfu of virus was administered intratumorally and CE activity was determined at the indicated time intervals.

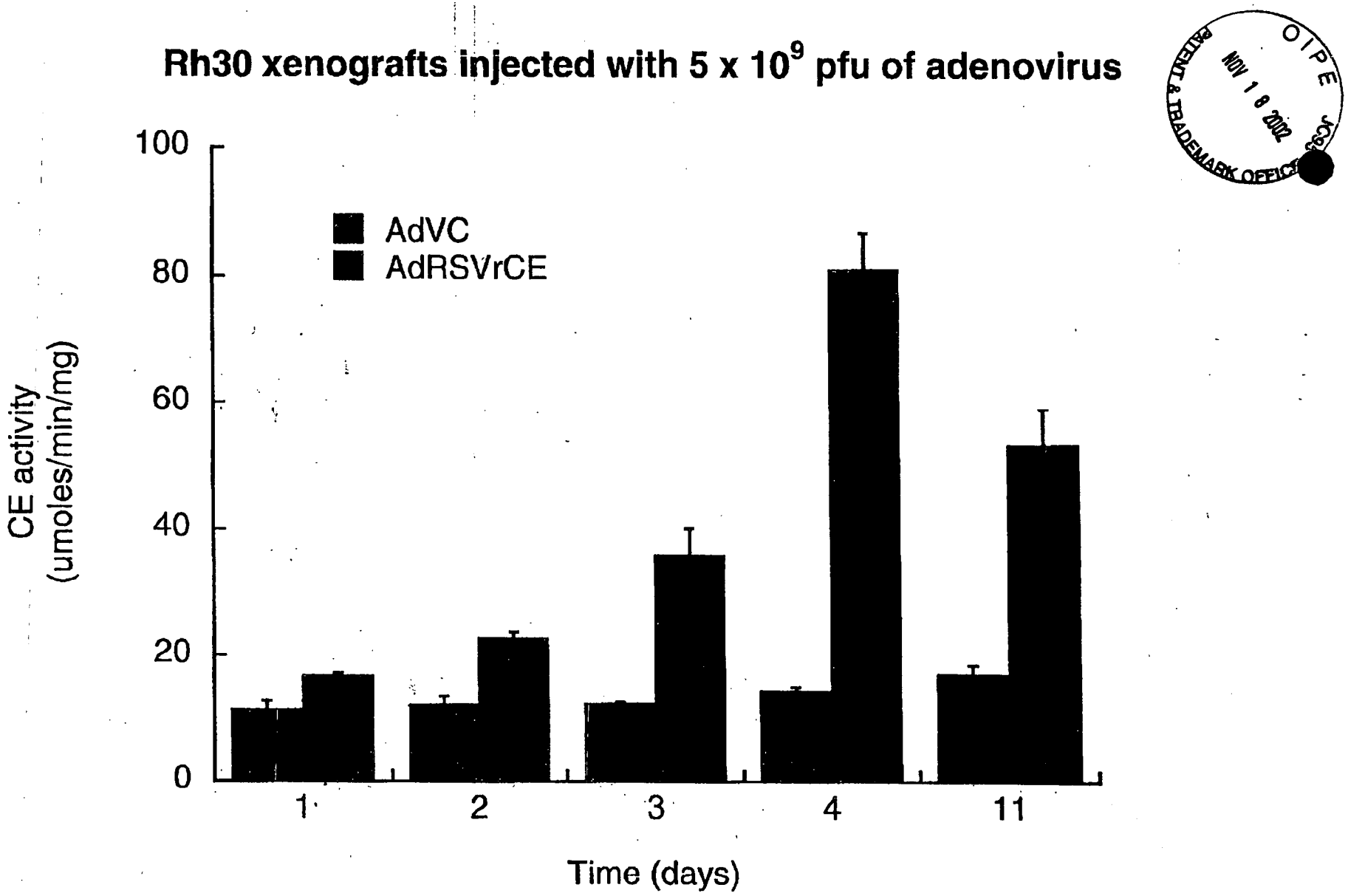
Figure 3. SN-38 concentrations in MB231 xenografts and serum of mice following repeat intratumoral administration of Onyx 713 adenovirus, and i.v. injection of 40mg/kg CPT-11. SN-38 concentrations were determined by HPLC 4 hours after CPT-11 injection.

Figure 4. Survival of nude mice bearing human C33-A xenografts following treatment with either CPT-11 alone, or with CPT-11 in combination with Onyx 713. Virus (4 x 10^8 pfu) was administered i.v. on days 1-5 and 19-23. CPT-11 (0.3mg/kg) was injected intratumorally on days 8-12 and 26-30.

Figure 5. CE activity in Rh30 xenografts transduced with either vector control adenovirus (Ad-VC), adenovirus containing the rabbit liver CE cDNA under the control of the CMV promoter (Ad-CMVRabfl), or adenovirus containing the rabbit liver CE cDNA under the control of the RSV promoter (Ad-RSVRabfl). 5 X 10^9 pfu of virus was administered intratumorally. CE activity was determined at the indicated times using o-nitrophenyl acetate as a substrate.

Figure 6. SN-38 concentrations in Rh30 tumor extracts following injection of either vector control adenovirus (Ad-VC), adenovirus containing the rabbit liver CE cDNA under the control of the CMV promoter (Ad-CMVRabfl), or adenovirus containing the rabbit liver CE cDNA under the control of the RSV promoter (Ad-RSVRabfl). SN-38 concentrations were determined at the indicated times.

FIGURE 1



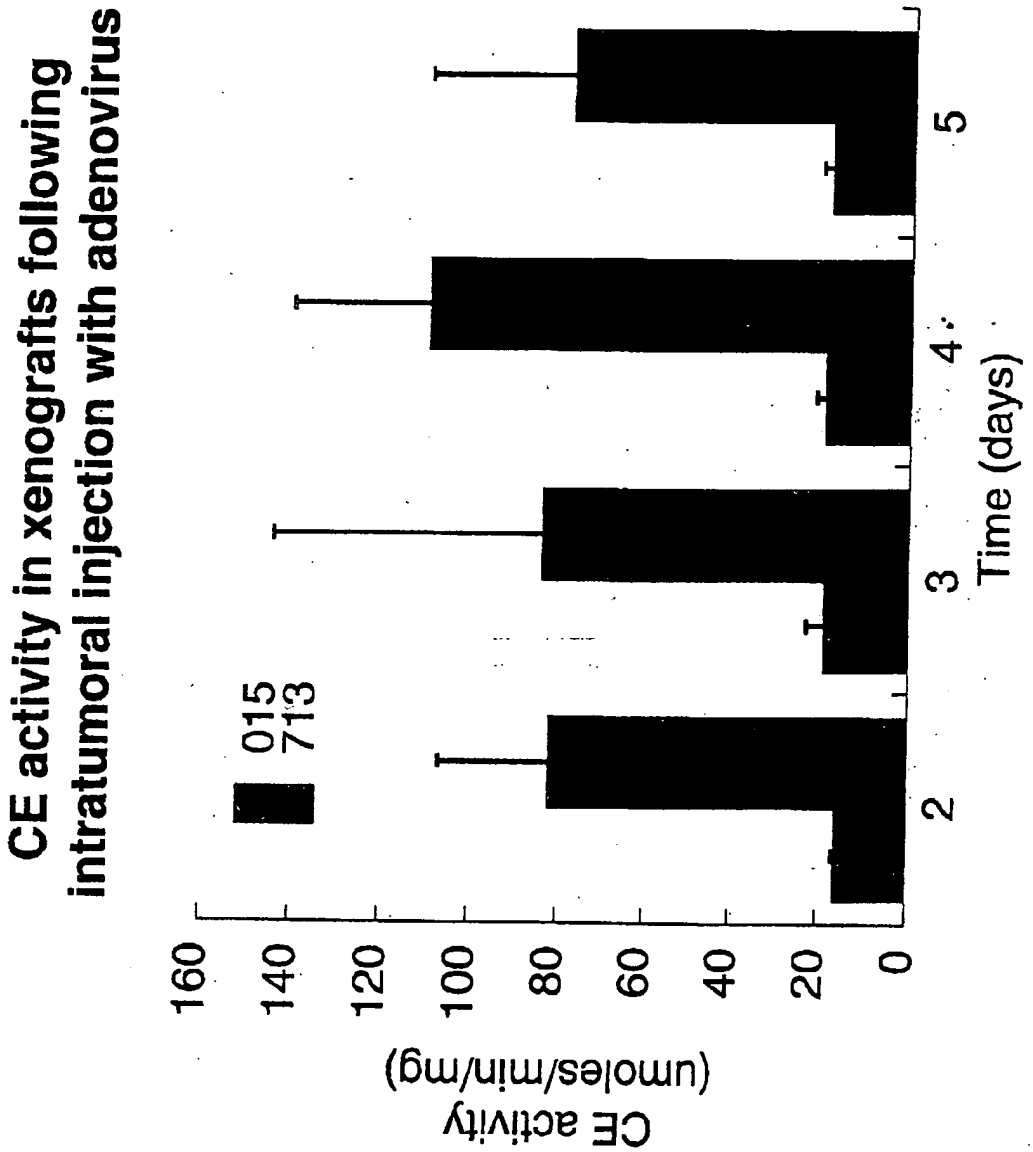


FIGURE 2

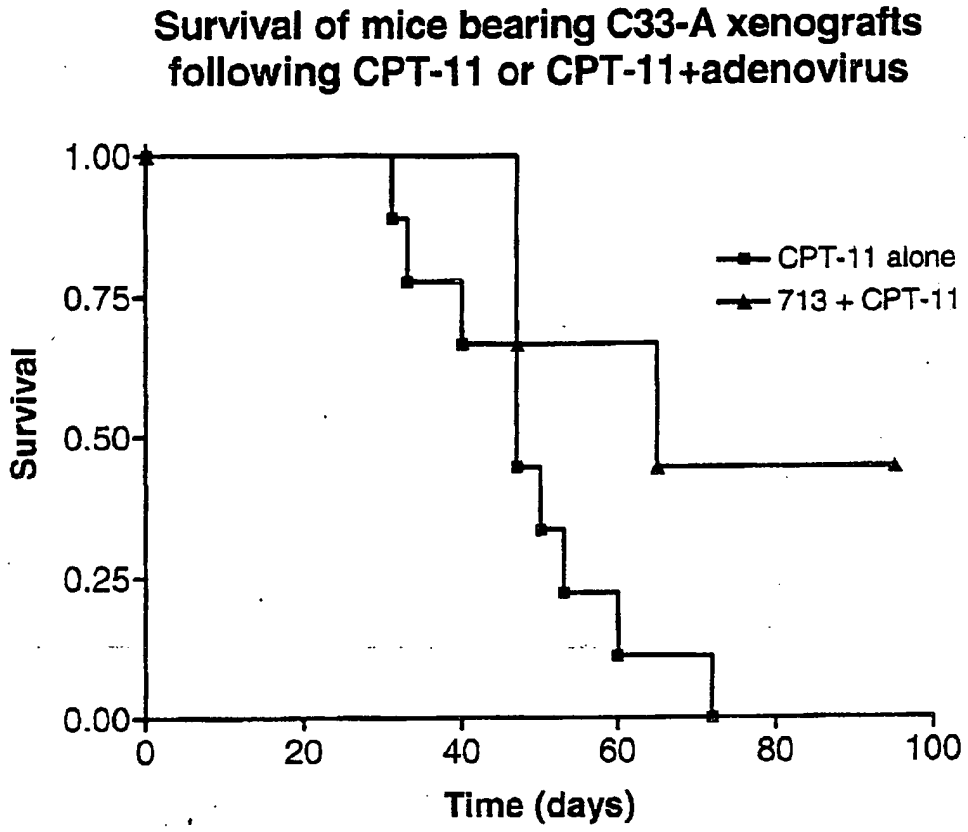


FIGURE 3

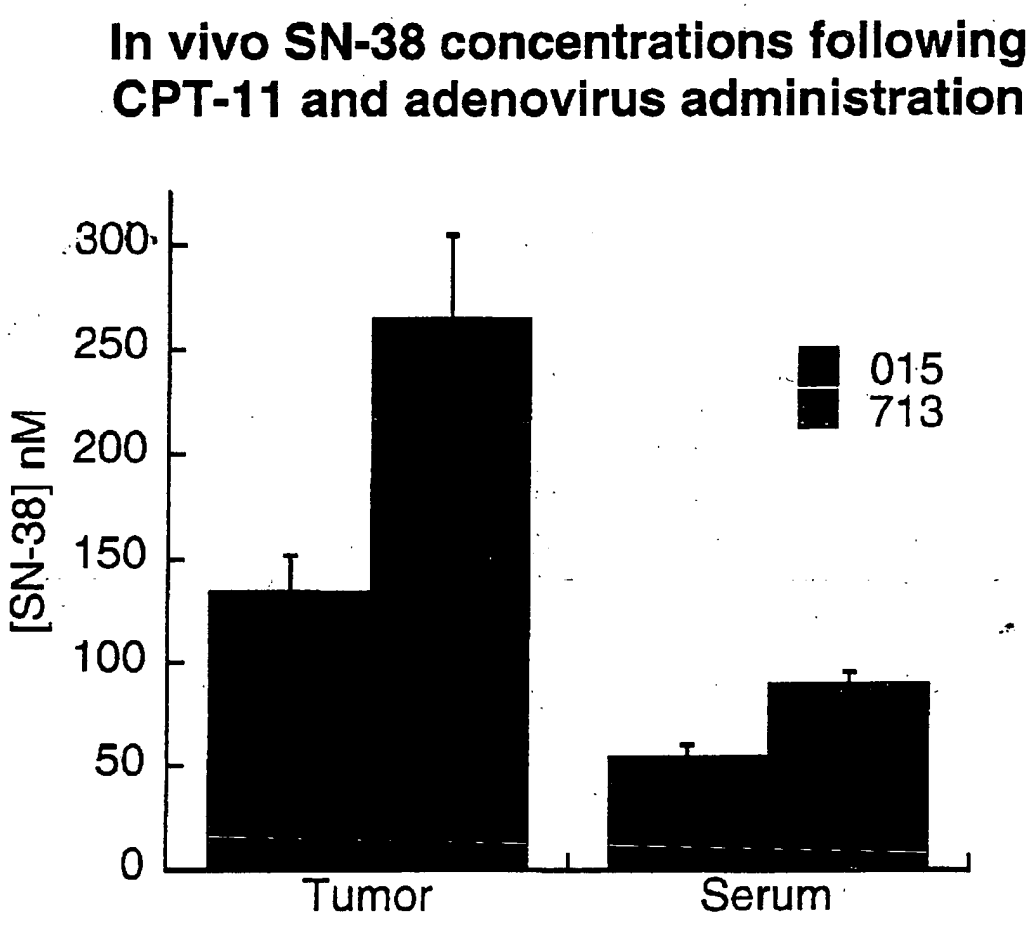


FIGURE 4

FIGURE 5

Rh30 Xenograft Transduced with Adenovirus ONPA Conversion 9/30/02

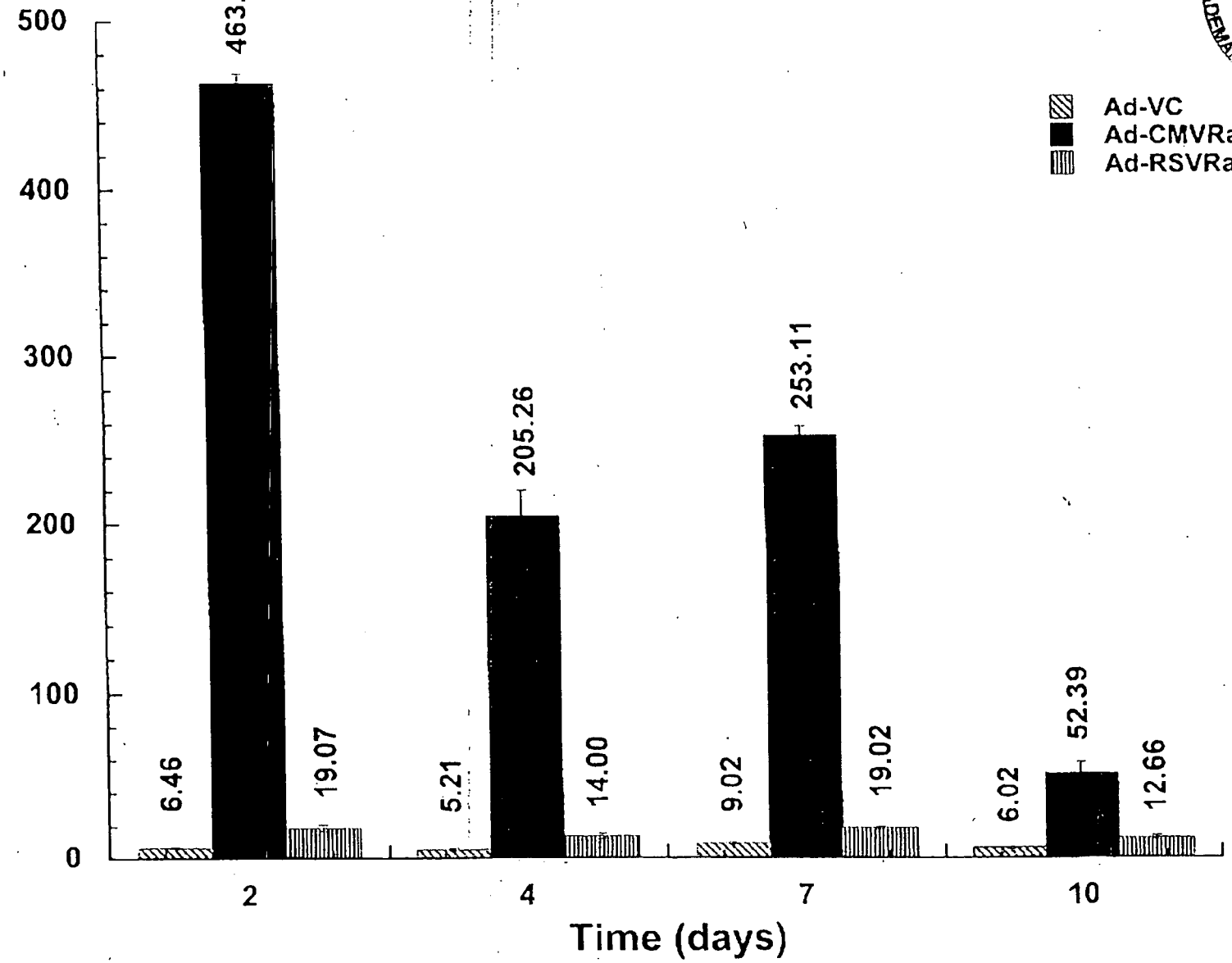


FIGURE 9